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Dr. ing. M.A. Gerritzen

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# Table of contents

# Preface

This report is a synopsis of four subsequent studies that have been conducted as part of the research project. Summaries of four separate studies are presented in Chapters 2 to 5. Based on the results of a literature review, the four studies and results from other relevant research projects, suggestions have been formulated on how to reduce the time to loss of consciousness (Chapter 6) and the expediency of slaughter procedures in terms of animal welfare (Chapter 7). The discussion and general conclusions have been formulated based on the results of the four individual studies (Chapters 2-5) and must be assessed within the context of the agreements as stated in the covenant between the Dutch government and the stakeholders on slaughter without stunning. In particular with regard to the agreement that the period during which the animal remains conscious after neck cutting should not exceed 40 s, the use of selected indicators to determine unconsciousness, and conditions for restraint of animals.

This report focusses on slaughter without stunning from an animal welfare perspective only without any qualification of the associated religious rites and perspectives, political decisions or societal opinions.

# Summary

The Dutch Parliament adopted a motion to enhance the welfare of animals being slaughtered (motion Ouwehand 22 June, 2011, TK 31 571 Nr. 14). In 2011 also an agreement (‘covenant’) between parties involved in unstunned ritual slaughter was achieved, in which certain areas were identified which require further investigation to safeguard or improve animal welfare during slaughter without stunning (Convenant onbedwelmd slachten volgens religieuze riten van 5 juni 2012, Stcrt. 2012, 13162).
In order to investigate the listed areas, the following research topics are considered relevant to slaughter without stunning in The Netherlands:

1. Identification of the current situation of slaughter without stunning in The Netherlands;
2. Validation of indicators to assess (un)consciousness;
3. Difference between restraining cattle in an upright or an inverted position;
4. Reducing time to loss of consciousness after neck cut;
5. The expediency of slaughter procedures in terms of animal welfare.

The current situation of slaughter without stunning in The Netherlands (1) was identified by performing observations of cattle slaughter in 27 different Dutch slaughter plants. These observations were performed by a researcher from Wageningen UR Livestock Research (WLR) and an inspector from the Dutch Food and Safety authorities (NVWA). Results from these observations were presented in WLR report 395.
Indicators to assess unconsciousness (2), referred to in the covenant on slaughter without stunning, were validated in two different studies, in sheep (WLR report 380) and in veal calves (WLR report 405). In these two studies reflexes and behavioural observations were compared with brain activity as recorded by an electroencephalogram (EEG). A comparative study of the responses of cows to upright and inverted restraining (3) were assessed under standardized conditions in a commercially available restraining box (WLR report 379). Information from these different studies was supplemented with information from an observational study in Dutch slaughter plants as part of the EU project ‘Borest’. This data and information was analysed to obtain insight into the possibilities for time reduction to loss of consciousness after neck cut without stunning (4) and to determine the expediency of slaughter procedures in terms of animal welfare (5).

The main conclusions concerning these 5 research questions can be summarized as follows;

*1. Identification of the current situation of slaughter without stunning in The Netherlands*
In Dutch slaughter plants, restraining methods for sheep, goats and cattle varied widely. Restraining methods did not meet legal requirements in a large proportion of observations.

*2. Validation of indicators to assess (un)consciousness*
Indicators to determine unconsciousness during slaughter without stunning are often difficult to apply due to the layout of the slaughter plant and/or the method of restraining. When restraint of the entire animal is applied, it is often impossible to observe the righting reflex and rhythmic breathing. Furthermore, rhythmic breathing cannot be observed by checking movements of the mouth or nostrils in animals slaughtered without stunning, since these movements are disrupted by severing of the trachea. In addition, the pain stimulus following the neck cut, though the animals were conscious based on EEG activity, did not yield an overt and reliable response in all animals. The same holds for the reaction to a threat. This reaction was often difficult to assess due to the amount of blood flowing over the eyes of inverted animals and was also found to be absent in “EEG-conscious” animals. The cornea-, or eyelid reflex was often still present when calves had already lost consciousness based on EEG activity. Presence of the eyelid-, or cornea reflex at 40 s therefore was not sufficient to establish that the animal was (still) conscious. Absence of the eyelid-, or cornea reflex on the other hand reflected unconsciousness in both sheep and cattle. Thus, neither the righting reflex, rhythmic breathing, the eyelid- or cornea reflex nor

the response to a pain stimulus or threat can be used in cattle and sheep as reliable indicators of unconsciousness under practical slaughterhouse conditions.

*3. Difference between restraining cattle in an upright or an inverted position;*

Restraint and rotation is stressful for cows, and it has been shown that there is an acute stress response following rotation. There were differences in indicators (i.e. presence of whites of the eyes or blood pressure) between cows in an upright position and 90 or 180 degrees rotation, but there was no overall indication that one of the treatments (upright and rotation to 90 degrees or 180 degrees) is more stressful to the cow than any of the other treatments. During upright restraint the quality of the bleeding appeared to be hampered more frequently than during inverted restraint, resulting in a significant delay in the induction of unconsciousness in animals remaining upright. In terms of animal welfare, stress due to restraining itself should be taken into account alongside the effects of each restraining method (e.g. quality of bleeding).

*4. Reducing time to loss of consciousness after neck cut;*

The duration to loss of consciousness after the neck cut varied strongly between animals and species. The majority of the sheep in the different studies were considered unconscious well before end of the 40 second period (agreed duration in the covenant). The majority of cattle, however, were considered to remain conscious for longer than 40 seconds. Since the bleeding quality in the majority of the animals was considered good, it can be concluded that changing the neck cut procedure is unlikely to reduce the duration to loss of consciousness in both sheep and cattle. A significant reduction in the conscious period can be achieved by applying post-cut stunning (i.e. captive bolt stunning).

*5. The expediency of slaughter procedures in terms of animal welfare.*

The expediency of slaughter without stunning for animals in terms of animal welfare was evaluated in the context of the multi-party agreement on slaughter without stunning in The Netherlands. Important criteria regarding animal welfare are; 1) that animals can be restrained according to regulations, 2) that animals are unconscious at 40 seconds post neck cut.

1) Although there are large differences in the quality of restraining methods and although the quality of restraining is, in practice, often inadequate, it is concluded that it is technically feasible to restrain all categories of animals in an acceptable manner according to legal requirements.
2) It is concluded that the vast majority of sheep and goats are unconscious within 40 seconds after the neck cut and therefore meet that set criterion. However, the majority of cattle (i.e. veal calves, cows and bulls) are still conscious at 40 seconds post neck cut. There is a large variation in the duration to loss of consciousness between animals and cattle can remain conscious for up to 2 minutes.

# Samenvatting

De Tweede Kamer heeft de motie 'Verbetering van het welzijn van slachtdieren' (motie Ouwehand, 22 juni 2011, TK 31 571, Nr. 14) aangenomen. Naar aanleiding van deze motie en de in het convenant onbedwelmd slachten volgens religieuze riten van 5 juni 2012 (Stcrt. 2012, 13162) opgenomen afspraken, zijn verschillende gebieden geïdentificeerd die verder onderzoek nodig hebben om het dierenwelzijn te handhaven of verbeteren tijdens slachten zonder bedwelming.

Om meer duidelijkheid te bieden binnen die genoemde gebieden, zijn de volgende thema's onderzocht die relevant zijn voor het slachten zonder bedwelming in Nederland:

1. Identificatie van de huidige situatie van het slachten zonder bedwelming in Nederland;
2. Validatie van indicatoren om bewustzijn/bewusteloosheid te beoordelen;
3. Verschil tussen fixeren in een rechtopstaande en omgekeerde positie van vee;
4. Het verminderen van de duur tot verlies van bewustzijn na de halssnede;
5. De geschiktheid van dieren onderworpen aan verschillende slacht procedures.

De huidige situatie van het slachten zonder bedwelming van herkauwers in Nederland (1) is in kaart gebracht door waarnemingen uit te voeren in 27 verschillende Nederlandse slachterijen. De waarnemingen werden uitgevoerd door een onderzoeker van Wageningen UR Livestock Research en een inspecteur van de Nederlandse Voedsel en Waren Autoriteit (NVWA). De resultaten van deze waarnemingen zijn reeds gepresenteerd in WLR rapport 395.
De indicatoren om bewusteloosheid te beoordelen bij het slachten zonder bedwelming (2) zoals die zijn afgesproken in het convenant werden gevalideerd in twee verschillende studies, één bij schapen (WLR rapport 380) en één bij vleeskalveren (WLR rapport 405). In deze twee studies werden reflexen en gedragsobservaties vergeleken met hersenactiviteit (elektro-encefalogram, EEG). De reactie van koeien op de fixatie in een rechtopstaande positie en gekantelde positie (3) werd onderzocht onder gestandaardiseerde omstandigheden in een commerciële kantelkooi (WLR rapport 379). In deze studie werden gedragsmatige en fysiologische parameters van koeien in een rechtopstaande of omgekeerde positie vergeleken. Naast de informatie uit deze studies, werd informatie uit een observationele studie verzameld in verschillende Nederlandse slachterijen, als onderdeel van het EU-project 'Borest', om zo inzicht te krijgen in de mogelijkheden de duur tot bewusteloosheid na de halssnede zonder verdoving terug te brengen (4). Daarnaast werd de geschiktheid van dieren onderworpen aan het slachtproces nader bekeken (5).

De belangrijkste conclusies van deze 5 onderzoeksvragen kunnen als volgt worden samengevat;

*1. Identificatie van de huidige situatie van het slachten zonder bedwelming in Nederland*
Op de bezochte slachthuizen in ons land bestaat een grote variatie tussen fixatiemethoden voor schapen, geiten en runderen. Bij een groot deel van de waargenomen slachtingen voldeed de fixatiemethode niet aan de vastgelegde eisen.

*2. Validatie van indicatoren om bewustzijn/bewusteloosheid te beoordelen;*
Alle vermeende indicatoren om bij dieren die zonder bedwelming geslacht worden, bewusteloosheid vast te stellen bleken in de praktijk nauwelijks bruikbaar door de opzet van de slachterij of de fixatiemethode. Door de volledige fixatie van de dieren is het meestal niet mogelijk de oprichtreflex en ritmische ademhaling te observeren. Bovendien kan een ritmische ademhaling niet worden gecontroleerd aan de hand van bewegingen van de bek of neusvleugels omdat deze bewegingen worden gemaskeerd doordat de luchtpijp is doorgesneden. Ook reageerde het merendeel van de dieren na de halssnede niet op een pijnprikkel in de neus of het oor. Deze dieren waren op basis van EEG activiteit echter nog wel bij bewustzijn. Bovendien blijkt de reactie op dreiging vaak lastig te beoordelen door de hoeveelheid bloed die over de ogen stroomt. Deze was dan ook bij een groot deel van de dieren afwezig, ondanks dat de

dieren volgens EEG activiteit bij bewustzijn waren. Tenslotte bleek de cornea- of ooglid reflex vaak aanwezig bij kalveren die op basis van hersenactiviteit (EEG) bewusteloos waren. Aanwezigheid van de ooglid- of cornea reflex op 40 s geeft dus niet weer of een dier nog bij bewustzijn/bewusteloos is. Afwezigheid van de ooglid- of cornea reflex anderzijds, gaf altijd bewusteloosheid weer in zowel schapen als runderen.

*3. Verschil tussen fixeren in een rechtopstaande en omgekeerde positie van vee;*
Fixatie en kanteling van dieren is stressvol en eerder onderzoek heeft aangetoond dat het kantelen een direct stress effect heeft. Er waren verschillen in indicatoren (zoals de aanwezigheid van oogwit en de bloeddruk) tussen koeien die rechtop stonden of 90 of 180 graden waren gekanteld. Echter, was er in het algemeen geen aanwijzing dat een van de behandelingen (rechtop of een kanteling van 90 graden of 180 graden) meer stress voor het dier veroorzaakte. In dieren die rechtop stonden, was de kwaliteit van het uitbloeden vaker slecht dan van dieren die gekanteld waren, wat resulteerde in een aanzienlijke vertraging in het induceren van bewusteloosheid bij deze dieren. Wanneer je dierenwelzijn in acht neemt, zal er dus rekening moeten worden gehouden met stress als gevolg van de fixatie, alsmede het effect van de fixatie op bijvoorbeeld de kwaliteit van het uitbloeden.

*4. Het verminderen van de duur tot verlies van bewustzijn na de halssnede*
De tijd tot het verlies van bewustzijn na de halssnede varieerde sterk zowel binnen als tussen diersoorten. In de verschillende onderzoeken waren de meeste schapen bewusteloos binnen de afgesproken 40 seconden. Een groot gedeelte van de runderen bleek echter pas het bewustzijn te verliezen na 40 seconden. Aangezien de kwaliteit van uitbloeden in het merendeel van de dieren goed was, kan worden geconcludeerd dat het eventueel wijzigen van de procedure rondom de halssnede de tijd tot het verlies van bewustzijn vermoedelijk niet zal verminderen. Een methode om de tijd tot het verlies van bewustzijn aanzienlijk te verminderen is het onvoorwaardelijk gebruik van een schietmasker onmiddellijk na toepassing van de halssnede.

*5. De geschiktheid van verschillende slacht procedures in relatie tot dierenwelzijn*
De vraag of dieren kunnen voldoen aan de verschillende voorwaarden die gesteld zijn in het kader van het convenant Onbedwelmd Slachten zijn nader onderzocht. Belangrijke criteria met betrekking tot het welzijn van dieren die hieruit naar voren komen zijn dat; 1) dieren kunnen worden gefixeerd zoals geëist volgens de voorschriften, 2) dieren bewusteloos zijn op 40 seconden na de halssnede.
1) Hoewel er grote verschillen waren in de kwaliteit van fixeren en de fixatie in de praktijk vaak niet toereikend is, kan worden geconcludeerd dat het technisch mogelijk is om alle categorieën dieren adequaat te fixeren en te voldoen aan de regels rondom fixeren.
2) Er kan worden geconcludeerd dat de overgrote meerderheid van de schapen en geiten bewusteloos was binnen 40 seconden na de halssnede, waarmee wordt voldaan aan de gestelde eis. De meerderheid van de vleeskalveren, koeien en stieren was nog bij bewustzijn op 40 seconden na de halssnede. Er is een grote variatie in de tijd tot het verlies van bewustzijn tussen dieren, en runderen kunnen tot 2 minuten na de halssnede nog bij bewustzijn zijn.

# 1 Introduction

Arising from the societal debate concerning the welfare of livestock before and during slaughter, the Dutch Parliament adopted a motion to enhance the welfare of animals being slaughtered (motion Ouwehand 22 June, 2011, TK 31 571 Nr. 14). Subsequently a discussion commenced on whether or not to prohibit slaughter without stunning in the Netherlands. Slaughter without stunning has not been prohibited, but the Dutch Minister of Economic Affairs, after close consultation with concerned parties, listed several areas requiring further investigation to safeguard or improve animal welfare during slaughter without stunning (St.crt. 2012, 13162). These areas include:

- Identification of critical control points;
- Validation of indicators to examine unconsciousness;
- Reduction in time between neck cutting and unconsciousness;
- Number of neck cuts required;
- Method and duration of restraint.

In order to improve knowledge concerning these areas, the following research topics are considered:

- Identification of the current situation of slaughter without stunning in The Netherlands;
- Validation of indicators to assess (un)consciousness;
- Difference between restraining of cattle in an upright or inverted position;
- Reducing duration to loss of consciousness after the neck cut;
- The expediency of different slaughter procedures in terms of animal welfare.

The first three research topics have been studied in more detail in four different research projects i.e. 1. The current situation of slaughter without stunning in The Netherlands (WLR report 395); 2. Difference between upright and inverted restraining in cattle (WLR report 379); 3. Validation of indicators to determine unconsciousness in sheep (WLR report 380) and 4. Validation of indicators to determine unconsciousness during slaughter of veal calves (WLR report 405). Gained knowledge from these studies together with information from an EU observational study in Dutch slaughter plants (EU project 'Borest') formed the basis for an investigation of into the possibilities for a reduction in the duration time to loss of consciousness after neck cutting without stunning and the expediency of slaughter procedure in terms of animal welfare.

# 2 Identification of the current situation of slaughter without stunning in The Netherlands

## 2.1 Introduction

In the covenant on slaughter without stunning, the Dutch government and other interested parties, have agreed that the implications of the covenant should be evaluated. The covenant considers that for improving animal welfare the following steps should be undertaken: monitoring the numbers of animals actually slaughtered without stunning, gaining more insight into the average duration (in seconds) from neck cut until loss of consciousness and the level of discomfort during restraint. In order to set these first steps in Dutch slaughter plants, a protocol without stunning was developed in co-operation with the NVWA (Dutch Food and Consumer Product Safety Authority) and Wageningen UR Livestock Research (WLR).

Information concerning slaughter procedures and numbers of animals slaughtered was collected by NVWA veterinarians prior to the observations performed in the slaughter plants. Slaughter plants were asked for approval to observe the slaughter procedures on regular slaughtering days. In preparation for these observational visits, NVWA veterinarians and researchers from WLR were instructed to reliably collect data following a uniform method at the various slaughter plants.
There are 76 registered slaughter plants in The Netherlands to perform slaughter without stunning. The numbers of animals slaughtered without stunning vary per slaughter plant from only a few to approximately 50.000 animals per year. Some slaughter plants only perform slaughter without stunning at specific times during the year i.e. in preparation for religious holidays. At present, without an obligation to register each animal slaughtered without stunning, the NVWA is unable to provide an accurate total number of animals slaughtered without stunning in the Netherlands annually.

## 2.2 Material and Methods

A total of 27 different slaughter plants were visited in the period from December 2013 to June 2014. In these slaughter plants, livestock is regularly or always slaughtered without stunning and they are representing the fast majority of animals slaughtered without stunning . At 20 of the 27 slaughter plants, a total of 165 cattle were observed varying in age from 6 to 119 months and from 84 to 1025 kg in body weight. In 22 of the 27 slaughter plants a total of 216 sheep and in one slaughter plant 9 goats were observed at/during slaughter/being slaughtered. These animals varied in age from 1.5 to 48 months and from 5 to 75 kg in body weight.
During the visits to the slaughter plants information on the following topics was collected:

1. Restraint: including entrance to the restraining box and facilities prior to restraint;
2. Bleeding out: the quality of bleeding out and registration of complications i.e. ballooning;
3. Assessment of indicators to validate (un)consciousness at 40 s after the neck cut.

Bleeding out and duration to loss of consciousness can be affected by method of neck cutting, location of the neck cut, number of cuts and the size of the knife blade. The following aspects of the neck cut were therefore registered:
Location of the neck cut in relation to the neck vertebrae (i.e. C1 to C6);
Number of cutting movements (one cutting movement was/is defined as a single uninterrupted movement during which the knife passes forwards and backwards/to and for once);
Quality of bleeding out was judged visually, based on blood flow;

Moreover, the presence (+), absence (-) or inability (0) to judge the indicators of consciousness were registered.

In the Netherlands it was formally agreed by the partners who have signed the covenant that animals subjected to slaughter without stunning should be unconscious within 40 s after starting the neck cut, based on absence of at least 3 of the following 5 indicators: 1. Reaction to threat reflex; 2. Reaction to a pain stimulus; 3. righting reflex; 4. (spontaneous) eyelid reflex; 5. rhythmic breathing. The palpebral and corneal reflex were added as additional indicators to be evaluated at 40 s post cut.

## 2.3 Results

Results of the observations are described for cattle, sheep and goats in more detail in WLR Report 395.

*Restraint:*
In 49% of the cattle slaughtered without stunning, quality of restraint was assessed to be insufficient or unacceptable. Insufficient or unacceptable restraint indicated that the body and/or head of the animal was held either too loosely or too tight. In 93% of the sheep slaughtered without stunning, quality of restraint was assessed to be insufficient or unacceptable. Insufficient or unacceptable restraint in sheep often referred to inadequate restraint of the head (mechanical or manual) directly after the neck cut.

*Neck cut and bleeding out:*
The majority of cattle and sheep/goats were cut in close proximity to the jaw, at the level of the first or second cervical vertebra (C1-C2). All cattle were slaughtered with 1 to 6 cutting movements. A single neck cut was sufficient in 42% of the cattle.
The number of sheep slaughtered with a single neck cut was 79%. Bleeding out was assessed to be unacceptable in 17% of the cattle and 14% of sheep.

*Indicators of (un)consciousness at 40 s:*

- Rhythmic breathing

Standardized evaluation of rhythmic breathing appeared to be difficult. The value of the evaluation of this indicator after severance of the trachea is disputable.

- Threat reflex

The threat reflex was difficult to perform in 12% of cattle and 67 % of sheep. Animals often had closed their eyes, either voluntarily or due to a tight head restraint or a high flow of blood over the eyes causing them to close.

- Blinking response and eye reflexes:

Spontaneous blinking was only observed in a small proportion of cattle. The eyelid or palpebral reflex and corneal reflex were not observed in 10% of cattle.
Spontaneous blinking was not observed in sheep. After the neck cut, sheep often had their eyes tightly closed. Both the palpebral and corneal reflex were not observed in more than 70% of the sheep.

- Righting reflex:

This reflex could not be evaluated when cattle or sheep were restrained, except for sheep placed in a V-shaped restrainer. Therefore, this indicator is of limited use under practical circumstances.

- Reaction to a pain stimulus (or withdrawal reflex):

This reflex could not be assessed in 22% of the cattle slaughtered without stunning. Uncontrollable head movements prevented the performance of a pain stimulus to the nose. In a majority of the animals, the withdrawal reflex was absent. Other reflexes, however, were often present at 40 s and based on these observations it remains uncertain whether or not animals were conscious at this stage.

# 3 Upright versus inverted restraint of cows

## 3.1 Introduction

Knowledge concerning the effect of current restraining methods on the welfare of cattle and small ruminants is limited, especially the impact of positioning and rotating the animal warrants further study. In addition, information is scarce concerning the methods and numbers of cattle, sheep and goats that are being restrained and rotated in practice. As a response to parliamentary discussions, the Ministry of Economic Affairs has commissioned a study into the methods of restraint used in The Netherlands, with special emphasis to the position of the animal (large and small ruminants) during restraint and rotation. The aim of the present study was to gain insight into the levels of stress animals encounter during different methods of restraining and rotating.

## 3.2 Material and methods

Impact on animal welfare of upright versus inverted restraining was assessed based on behavioural and physiological indicators. The experiment was conducted at a test facility for standardisation of the procedure and involved nine dairy cows. During the experiment, all nine cows were exposed to three different treatments following a 3x3 Latin square design. Treatments included: 1. Restraining upright without rotation; 2.Restraining followed by 90 degrees rotation; 3. Restraining followed by 180 degrees rotation (inverted position/dorsal recumbence). Figure 1 shows the a cow being rotated. The rotation phase lasted up to three minutes. The animals were allowed for one week to recover between treatments.



***Figure 1****: A cow being rotated*

To measure physiological indicators all cows had a surgically implanted telemetry transmitter allowing registration of body temperature, activity, blood pressure and cardiac activity (ECG) during the experimental period. In order to measure behavioural indicators, cows were equipped with a non-invasive sensor to detect rumination, eating and drinking behaviour and activity was recorded by 3-dimensional accelerometers, commonly referred to as pedometers. Behaviour was also scored when driving the animals towards the restraining pen. Behavioural observations included: number of vocalisations, refusal to move, displaying whites of the eyes, posture, defecating, urinating, turning or escape attempts, and amount of persuasion needed to stimulate the cow to move from one area to another. In order to study the effect of treatment on recovery of the animal, laying and rumination activity were also monitored.

## 3.3 Results

Results indicate a significant increase in both time and amount of stimulation needed to drive the animals out of a starting pen towards the restraining pen once they previously experienced being restrained. Animals that were not rotated after being restrained (0 degrees rotation) displayed less whites of their eyes compared to animals that were rotated to 90 or 180 degrees. Heart rate patterns over time differed significantly between treatments. Heart rates of animals restrained without rotation varied between 88 and 95 beats per minute, while heart rates of animals restrained with 90 or 180 degrees rotation were lower, about 76 to 80 beats per minute. While there was no effect of treatment on the pattern of diastolic blood pressure during the three minutes of treatment (rotation 0, 90, 180 degrees) there was a significant effect of earlier experience in the restraining pen. Animals with prior experience in the restraining pen (in this experiment) showed a significant drop in diastolic blood pressure over the period of three minutes in the restraining pen, regardless of the treatment. There was no effect of treatment on either latency to laying or ruminating, nor was there an effect of treatment on the length of the first laying or rumination bout after release of restraint.

## 3.4 Conclusions

The results of the current study indicate that restraint and rotation to 90 or 180 degrees, as is standard practice for slaughter without stunning, is stressful for cows. The findings indicate that there is an acute stress effect from restraining, from rotation, and that repetition of restraining and rotation is more stressful for the animal. Although certain physiological indicators differed between treatments, there is no overall indication that one treatment (upright, 90 degrees or 180 degrees) is more stressful than the other.

# 4 Validation of indicators used to assess unconsciousness during slaughter in sheep

## 4.1 Introduction

New Dutch legislation requires that unconsciousness should be tested at 40 s post neck cut in animals subjected to slaughter without stunning. Animals that are not unconscious at 40 s following neck cut should be stunned immediately. Generally, assessment of unconsciousness in animals at slaughter plants is performed on the basis of behavioural indicators (e.g. reflexes and voluntary movements). In experimental set-ups, the use of brain activity as represented in an electroencephalogram (EEG) is considered an objective alternative. The validity and applicability of most behavioural indicators for assessing unconsciousness under different slaughter circumstances (with and without stunning) are under (inter)national debate and need further investigation (Part B). In order to study absence of indicators that reflect unconsciousness, anaesthetic agents can be used to induce different stages of unconsciousness and allow for recovery, where stunning and exsanguination will lead to rapid and irrecoverable death of the animal. The use of anaesthetic agents can therefore provide a model to validate absence of the different indicators in the assessment of unconsciousness, and can provide detailed information on relations between these indicators and EEG activity (part A).
The aim of this study (part A and B) was to validate the following indicators to assess unconsciousness in sheep: rhythmic breathing, eyelid reflex, reaction to threat and withdrawal reflex (by pinching the ear). During part A of the study, the righting reflex was also tested.

## 4.2 Material and methods:

During part A of the study, mixed breed ewes (n=10, 36 ± 4 kg) were anaesthetised twice, six days apart, for ± 30 minutes after which they regained consciousness. The anaesthetic agent Propofol was used to achieve different levels of (un)consciousness. After recording an EEG baseline of five minutes, sheep received a bolus injection of propofol at T=0 min (8 mg $kg^{-1}$, intravenously) followed by continuous infusion of 8 mg $kg^{-1}$ $hr^{-1}$ for 28 minutes using an infusion pump. Administration of propofol was stopped at T=31 min, where after sheep regained consciousness. Reflexes were tested every two minutes. Respiratory rate (RR) and EEG were recorded continuously. State of consciousness was based on visual inspections of the EEG, as well as on analysis of derivatives of the EEG. Visual inspections of the EEG provided five different stages of consciousness, observed as: baseline, transitional (induction), unconscious, transitional (recovery) and recovery (similar to baseline).
During part BB of the study, mixed breed ewes (n=21, 38 ± 4 kg) were exsanguinated by neck cut, severing both jugular veins and carotid arteries. Reflexes were tested every two seconds. RR and EEG were recorded continuously. Loss of consciousness was based on visual assessment of the EEG. Visual assessment of the EEG provided three different stages of consciousness observed as: baseline, unconscious and iso-electric line (flat).

## 4.3 Results:

During part A, sheep lost and regained consciousness after propofol administration (T=0 min) at 00:43 ± 00:06 min and 28:27 ± 06:20 min respectively, based on visual assessment of the EEG. Breathing was rhythmic (based on visual assessment of the RR), from 01:26 ± 0:21 min until 21:13 ± 05:46 min, whilst animals were unconscious. Reaction to threat- and withdrawal reflexes were absent after 01:57 ±

0:31 and 02:48 ± 01:14 min and returned at 28:51 ± 06:14 and 13:36 ± 05:02 min, respectively. The eyelid reflex was absent at 04:40 ± 02:11 and returned at 10:45 ± 05:31 min, but did not disappear in 8 out of 20 observations. . Based on the EEG, however, all sheep were considered unconscious during that time. Pearson correlations, showed strong and highly significant correlations between visual assessment of the EEG and derivatives of the EEG with correlations ranging from r=0.53 to r=0.97 (P<0.01), supporting the classification of different stages of unconsciousness as found in visual assessment of the EEG. Significant and strong correlations found between EEG stages and behavioural indicators consisting of: 'transitional$_{rec}$' and 'recovery' with 1. Reaction to threat (r=0.58, P <0.01 and r=0.63, P <0.01); 2. righting reflex (r=0.78, P <0.001 and r=0.81, P <0.001) and 3. Return of non-rhythmic breathing (r=0.81, P <0.001 and r=0.82, P <0.001). This indicates only the presence of an reaction to threat, presence of righting reflex and change in breathing pattern were potential indicators of animals regaining consciousness after propofol anaesthesia. Variation in the withdrawal- and eyelid reflexes were difficult to interpret in the assessment of consciousness.

During part B, sheep lost consciousness 00:15 ± 00:04 min following neck cut (T=0 min), based on visual assessment of the EEG. The EEG was evaluated as being iso-electric, indicating brain death, at 00:27 ± 00:08 min. Irregular breathing was observed in all animals from 00:43 ± 00:12 min onwards. A reaction to threat was present (until 00:07 ± 00:01 min) in seven sheep, prior to loss of consciousness. No withdrawal reflex was seen following neck cut, during exsanguination. However, an eyelid reflex was observed in all sheep up to 01:14 ± 00:17 min post neck cut.

## 4.4 Conclusions:

The absence of the reaction to threat and the withdrawal reflex following neck cutting, indicated that absence of these indicators does not necessarily indicate unconsciousness. When absence of regular breathing or the eyelid reflex was observed, animals were always unconscious (as judged by EEG). However, these indicators were lost long after 40 s following neck cutting in some animals and it is therefore proposed to look for other, more accurate indicators of unconsciousness at 40 s following neck cutting.

# 5 Validation of indicators to determine unconsciousness during slaughter of veal calves

## 5.1 Introduction

One of the areas of concern raised in the motion (Ouwehand 22 June, 2011, TK 31 571 Nr. 14) on “Improving the welfare of livestock designated for slaughter” involves the assessment of unconsciousness in both stunned and unstunned animals. Furthermore, determination of unconsciousness at slaughter is mandatory according to EU Council Regulation 1099/2009. However, the validity of certain behavioural indicators to assess unconsciousness under different stunning and slaughter conditions is under (inter)national debate.
The aim of this study was to validate the absence of the righting reflex, rhythmic breathing, threat-, withdrawal-, cornea- and eyelid reflexes as indicators to assess unconsciousness in veal calves subjected to different stunning and slaughter methods.

## 5.2 Material and methods:

Eighty-two young calves (201 ± 22 kg) were randomly assigned to one of the following four treatments:

1. Captive bolt stunning followed by neck cut in an inverted position (n=25);
2. Slaughter without stunning in an upright position (n=7);
3. Slaughter without stunning in an inverted position (180° rotation) (n=25);
4. Slaughter without stunning in an upright position followed by captive bolt stunning at 40 s post neck cut (n=25).

Each calf was equipped with non-invasive EEG electrodes prior to the procedure. All reflexes were verified once before the treatments started. After the start of the procedure (T=0 s) reflexes were assessed at T=5 s for treatments 1 and 4 and from T=15 s every 20 s for all treatments until all reflexes showed a negative response three times in a row and a flat EEG was observed. Visual assessment of EEG traces was used to determine loss of consciousness. Timing of loss of consciousness was related with timing of loss of reflexes.

## 5.3 Results

Based on EEG activity, loss of consciousness was confirmed in all calves immediately after captive bolt stunning. The threat-, withdrawal,- cornea,- and eyelid reflexes were all absent post stunning.
Captive bolt stunning following a neck cut results in immediate loss of consciousness and is as effective in producing unconsciousness in veal calves as pre-cut captive bolt stunning.
All calves slaughtered without stunning in an upright position were considered unconscious at, on average, 109 s post neck cut. Calves slaughtered without stunning in an inverted position lost consciousness on average at 49 s post neck cut.
In this study, slaughter without stunning in an upright position resulted, on average, in a delay of 60 s before loss of consciousness compared with unstunned slaughter in an inverted position. The threat- and withdrawal reflexes were lost before calves were considered unconscious. The eyelid- and cornea reflexes, on the other hand, were lost after calves were considered unconscious. Hence these latter parameters appeared to be rather conservative indicators of unconsciousness in non-stunned slaughtered calves since they were observed until 76 ± 50 s and 85 ± 45 s (mean ± SD) after calves were considered unconscious. Rhythmic breathing and the righting reflex could not be assessed because the animals were fully restrained in a rotatory box.

## 5.4 Conclusions

The righting reflex is not a feasible indicator to assess unconsciousness while animals are fully restrained in a stunning box. Since restraint is mandatory until the animals are unconscious, the righting reflex should not be used to assess state of (un)consciousness during unstunned slaughter.
Rhythmic breathing can't be assessed in an objective way in animals with a severed trachea, restrained in a stunning box. Therefore, under the above mentioned conditions, rhythmic breathing is not considered to be a feasible applicable indicator to determine(un)consciousness at slaughter without stunning.
Absence of a reaction to a pain stimulus did not indicate unconsciousness. Moreover, the majority of the animals did not respond to a pain stimulus immediately following the neck cut when they were considered to be EEG-conscious.
Reaction to a threat is often difficult or impossible to determine due to the amount of blood flowing over the eyes of inverted animals, and a response to a threat was also absent in EEG-conscious animals.
The cornea-, or eyelid reflex was often (still) present when/until after calves had lost consciousness/were considered EEG-unconscious, but when absent they indicate unconsciousness in calves subjected to slaughter without stunning. Therefore, both the eyelid- and the cornea reflex provide a very conservative confirmation of loss of consciousness.

# 6 Reducing time to loss of consciousness in animals subjected to slaughter without stunning

## 6.1 Introduction

European legislation provides laws, rules and procedures regarding the slaughter of livestock (Council Regulation (EC) 1099/2009, Council Directive 93/119 1993, GWvD 1992). Article 4 of Council Regulation (EC) No 1099/2009 dictates a mandatory pre-slaughter stunning, with the exception of particular methods of slaughter prescribed by religious rites, to ensure unconsciousness and insensibility in order to prevent unnecessary suffering of animals. There is no consensus on the extent to which slaughter of conscious, meaning sensible and/or aware, animals causes them pain and distress. It is claimed that when a clean incision is made with an exquisitely sharp knife, significant pain and distress are avoided. It is suggested Grandin 1994, Rosen 2004; Johnson et al. 2012) that massive stimulation of all sensory nerves after the neck cut may lead to shock and distress that would be experienced as pain for the duration of consciousness. Until now, neurophysiological methodologies have not provided the ultimate answer to this issue. However, with the discussion regarding pain perception during slaughter in mind, it was formally agreed in the Netherlands that animals subjected to slaughter without stunning should be unconscious within 40 s to reduce pain and distress. Moreover, it was agreed that an effort should be made to a reduction in the duration between the neck cut and time to loss of consciousness.
This part of the research project aimed to identify the possibilities to reduce the time to loss of consciousness after the neck cut in animals subjected to slaughter without stunning.

## 6.2 Time to loss of consciousness after the neck cut

### 6.2.1 Sheep

In the experiment on '*validation of indicators to assess unconsciousness in sheep*' (WLR Report 379), sheep were considered unconscious at 15 ± 4 sec post neck cut, based on EEG activity (Figure 2). These findings are similar to studies by Devine et al. (1986) and Gregory and Wotton (1984) who observed loss of consciousness to occur at 14 ± 7 sec (n=10) and residual consciousness at 14 ± 5 sec (n=20) post neck cut respectively. The EEG became iso-electric at 27 ± 8 sec, which was comparable to findings of Newhook and Blackmore (1982) who reported that the sheep EEG was iso-electric (n=5) from 33 ± 13 sec post neck cut.

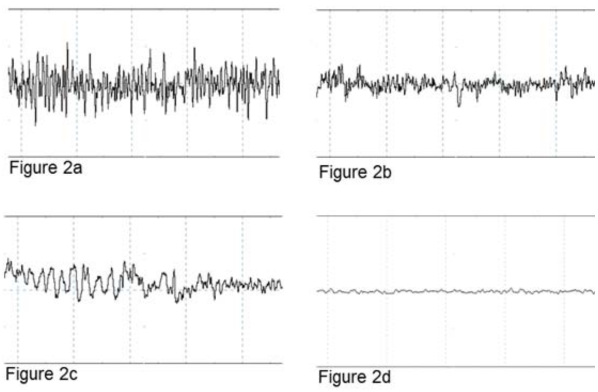



***Figure 2:*** *Typical examples of the different stages identified with visual assessment of EEG activity prior to and after stunned and unstunned slaughter in sheep. The four stages are: baseline (2a), transitional (2b), unconscious (2c), and minimal brain activity (2d).*

The neck cut was performed in all sheep by the same skilled halal slaughter man. Location of the neck cut did not differ between sheep in the 'sheep experiment' and was always close to the jaw at the location of the first cervical vertebra (C1).
In the observational study at 22 Dutch slaughter plants (WLR Report 395) the majority of the sheep were cut at location C1 or C2 with one or two cutting movements. In that study the quality of the neck cut was good in 86% of the observed sheep (n=216). In 14% of the sheep, however, bleeding quality was insufficient. Insufficient bleeding in sheep following a neck cut often results from a superficial cut or obstruction of the wound due to inappropriate restraint. Assessment of loss of consciousness based on absence/presence of reflexes indicated large variation between individual sheep. The percentages of sheep displaying a positive response to the following indicators of (un)consciousness at 40 s after the neck cut were: rhythmic breathing, 3%; spontaneous eye blinking, 0%; reaction to treat, 2%; reaction to a pain stimulus, 6%; palpebral reflex, 70% and cornea reflex, 77%.

### 6.2.2 Cattle

Results from the study on '*validation of indicators to determine unconsciousness in veal calves*' (WLR Report 380) indicated that time to loss of consciousness depended on body position (upright or inverted) during bleeding.
Blood flow was restricted in some of the calves slaughtered upright, at the moment that the position of the head temporarily obstructed the flow of blood from the vessels in the neck.
At 40 s post neck cut, none of the calves slaughtered in the upright position were unconscious. In the inverted position only 46% of the calves were unconscious at 40 s post neck cut. The moment when all calves were unconscious was 140 s post cut in the upright position and 109 s in the inverted position.
The findings are comparable to results described by Johnson et al. (2015) indicating that cattle may remain conscious for up to 85 s. Some studies even suggest loss of spontaneous brain activity in cattle at 75 ± 48 s post neck cut (range 19-113 s) and possible intermittent recovery of consciousness for 123 to 323 s after slaughter (Daly et al. 1988; Newhook and Blackmore, 1982).
In all the research performed within this project, EEG's were measured with minimal interference of the normal procedure at the slaughter plant. Electrodes were placed in an elastic halter with non-invasive electrodes on the skin. Furthermore, the neck cut was performed close to the jaw at location C1-C2 l by two skilled slaughter men, employed by the slaughter plant. It is therefore assumed that time to loss of consciousness was not increased due to the experimental procedure.
During observations at 20 Dutch slaughter plants (WLR Report 395) plus three at two different slaughter plants (EU project "Borest") a total of 315 cattle including veal calves, cows, and bulls were observed. For the majority of the animals (± 75%) the neck cut was performed at location C1-C2, as close as possible to the jaw. In 10% of the animals, the neck cut was performed at location C4 location or lower. In 58% of animals, 2 or more (up to 6) cutting movements were applied. In 17% of the observed cattle bleeding out was judged as insufficient. Main reasons for insufficient bleeding in cattle were an incomplete neck cut, where both arteries were not cut at once, obstruction of cut arteries due to ballooning, false aneurysms or blood clotting (Gregory et al. 2006). There was a lot of variation in loss of indicators of unconsciousness between cattle. At 40 s post neck cut rhythmic breathing was still present in 19% of the animals, spontaneous eye blinking in 20%; reaction to threat in 17%; reaction to pain stimuli in 19%; palpebral reflex in 87% and cornea reflex in 89% of the animals.

## 6.3 Measures to reduce duration to unconsciousness

Time to loss of consciousness in animals slaughtered without stunning depends on quality of bleeding as stated previously.
When major blood vessels supplying the brain are severed a catastrophic drop in cerebral blood flow results in unconsciousness and eventually brain death (Johnson et al. 2014). In non-stunned animals the lack of oxygen due to the loss of blood flow to the brain causes the onset of insensibility (Mellor & Littin 2004). Duration to unconsciousness can be affected by the method of neck cutting (Grandin 2012). Cutting close to the jaw bone, i.e. at location of C1, is supposed to reduce the incidence of ballooning or false aneurysms (Gregory et al. 2012)

Most In observed sheep and cattle slaughters animals were cut close to the jaw at C1-C2 resulting in a good quality bleeding out. Reduction of the conscious period by improvement in bleeding out quality due to location of the neck cut (i.e. closer to the jaw) is not considered help to further effective in reduction inthe duration to unconsciousness.
It is crucial that the neck cut is performed well, that the cut is deep enough and that obstruction of blood flow is solved immediately. However, Johnson et al. (2015) stated, that occlusion of the rostral stump of the carotid artery could result in the maintenance of brain perfusion. Occlusion of the arterial stump, a relatively common occurrence in some species, is characterised by retraction and contraction of the elastic portion of the arterial wall and thrombus formation around the severed end of the vessel (Gregory et al. 2010, Gregory et al. 2006, Gregory et al. 2012). Animals can routinely survive the occlusion of one common carotid artery (Clendenin & Conrad 1979) and so it is likely that a single occluded stump can result in continued brain perfusion until sufficient blood is lost to cause a drop in the systemic arterial blood pressure and therefore facilitates a delay in loss of consciousness. This necessitates that when bleeding is blocked or hampered, a second immediate intervention is required to open the blood vessels without delay.
The method and quality of restraint also appears to have an effect on bleeding rate (Chapter 5 en WLR Report 405). Pinching off the neck, closing the wound due to the position of the head or inadequacy in the second intervention when blood flow is obstructed has a negative effect on blood flow and thus extends the conscious period. Therefore, good quality restraining allows any emergency intervention(s) until unconsciousness has been confirmed.

Various slaughter plants, in The Netherlands, perform stunning immediately after neck cutting on cattle, especially veal calves. Application of this procedure guarantees that the bleeding starts in conscious, non-damaged animals, and that unconsciousness is established by (mostly captive-bolt) stunning very quickly after the neck cut. In the study on '*validation of indicators to determine unconsciousness in veal calves*' (WLR Report 380) one group of animals was slaughtered without stunning followed by post cut captive bolt stunning. From the results it became clear that post cut stunning resulted in immediate loss of consciousness. Therefore, it can be concluded that the conscious period can be reduced to a period equivalent to as long as it takes to perform the stun after the neck cut. Under practical conditions, during the observations for the EU project "Borest", it appeared that trained slaughter men can apply captive bolt stunning within a few seconds after performing the neck cut when cattle are properly restrained.

# 7 Expediency of animals to different slaughter procedures in terms of animal welfare

In order to safeguard animal welfare during slaughter, it is evident that the slaughter technique and equipment used as well as the layout of the slaughter site should be adapted to facilitate the type of animals that are being slaughtered. This implies that animals should be handled with care, that the type and size of the restraining box matches the size of the animals, that the knife used is suitable for the size of the animals, and that adequate emergency procedures are available.
The slaughter of animals without prior stunning is regulated by Article 4 of European Regulation (EC) No 1099/2009. Slaughter without stunning induces gradual loss of consciousness and consequently death due to the deprivation of nutrients and oxygenated blood to the brain and onset of brain ischemia. According to the European Regulation (Article 7), slaughter plant employees that perform slaughter without stunning are required to have a certificate of competence. Furthermore, the 'personnel' of the slaughter plant have to carry out systematic checks to ensure that the animals do not display signs of consciousness (article 4) before being released from restraint. The European Regulation also stipulates instructions for restraint (article 8) and the use of restraining equipment (article 9 and 15) when slaughter without stunning takes place.
The European Regulation also specifies that carcass dressing shall always begin after the onset of death. This also applies to animals subjected to slaughter without stunning.

Various aspects of the various slaughter techniques have to be assessed in order to determine which techniques are expedient to animal welfare. Furthermore, the decision as to which type of animal matches the conditions of slaughter is based on agreements stated in the covenant on slaughter without stunning according to religious rites (St.crt. 2012, 13162). Conclusions on this subject are based on the observations of the current situation of slaughter without stunning in the Netherlands (WLR Report 395), the experiment on restraint of ruminants (WLR Report 379), and the validation of indicators to determine unconsciousness in sheep and veal calves (WLR Report 380 and 405). The following paragraphs discuss whether or not animals meet the criteria as set in the covenant and which changes should be made in order to meet the criteria. Addressing issues such as: can adequate restraint be accomplished?; Does the size of the animals match the restraining equipment?; Can adequate neck cut and bleeding out be assured?; Are there emergency procedures available?; Is it possible to determine unconsciousness based on the agreed indicators and is consciousness lost within 40 s following the neck cut?

## 7.1 Sheep and goats

### 7.1.1 Restraint

Sheep slaughter plants in The Netherlands use a variety of different restraining methods during slaughter without stunning. Restraining methods vary in providing options for restraining animals in different body positions i.e. upright restrained on a V-shape conveyor belt (Figure 3), restrained in lateral position (one side) or in dorsal position (on the back).



***Figure 3**: V-shape conveyor belt*

The survey of Dutch slaughter plants showed that in 93% of the observations sheep restraining was inadequate or unacceptable. The main reason for inadequate restraint was that the head was no longer restrained directly following neck cut. Therefore, it is concluded that current restraining methods and/or mode of operation are not in line with regulations. However, the findings do not imply that sheep and goats are unfit for slaughter without stunning. It is expected that restraining methods and modes of operation can be adapted so that sheep and goats can be adequately restrained.

### 7.1.2 Bleeding

Most (>85%)sheep and goats were slaughtered using a single neck cut resulting in good quality bleeding. However, the quality and duration of bleeding, can be influenced by the quality of restraint. Most sheep were able to move their heads or rest their heads on an animal in front of them (in V-shaped conveyor belt) occasionally resulting in a restricted blood flow and thus inferior bleeding quality. Our findings also indicate that good quality bleeding out of sheep and goats can be achieved with a single cutting movement, in accordance with regulations and the covenant on slaughter without stunning.

### 7.1.3 Unconsciousness

According to covenant agreements the determination of unconsciousness should be based on the absence of at least 3 of the following indicators: rhythmic breathing; (spontaneous) eye blinking; reaction to threat; righting reflex and reaction to a pain stimulus (pinching the nasal septum). Often other studies have used the absence of the eyelid-, palpebral-, and cornea reflex to assess unconsciousness(Verhoeven et al. 2015, von Holleben et al. 2010, EFSA 2004, EFSA 2014, Velarde et al. 2013). When examining the indicators agreed upon in the covenant, it appears that at 40 s post neck cut, rhythmic breathing is absent in almost all sheep and goats. Spontaneous blinking and the reaction to a threat could often not be observed due to tight closure of the eyes. The righting reflex was absent in most sheep at 40s and a reaction to a pain stimulus was absent in almost all animals at 40 s after the neck cut. This means that at least 3 of the 5 indicators were absent at 40 s after neck cutting and thus animals were expected to be unconscious at that point. The EEG data in the experiment on validation of indicators to assess unconsciousness in sheep (see Chapter 4 and WLR Report 380) confirmed that most sheep were unconscious well before 40 s post neck cut.
The palpebral reflex and cornea reflex appeared to be present for a much longer period of time, also after loss of consciousness was observed. At 40 s post neck cut, the palpebral reflex was present in 70% of the animals and the cornea reflex was present in 77% of the animals. The palpebral and cornea reflexes are concluded to be fairly conservative indicators of unconsciousness. When absent, the animals are most likely to be unconscious.

## 7.2 Cattle

### 7.2.1 Restraining

In The Netherlands ,there is large variety between cattle slaughtered without stunning in age and size. Importantly, most slaughter plants that slaughter cattle varying in size only have one or two restraining boxes (Figure 4). The large variation in animal size requires special attention regarding restraint before the neck cut. The observational study in Dutch slaughter plants (Chapter 2, WLR Report 395) showed that upright and rotating restraining boxes cannot be adjusted in order to adapt to the wide range in animal size (i.e. veal calves, young and mature bulls, dairy cows and beef cattle. In approximately 50% of cases, animals were not restrained as required by legislation. Either the head, body or both were held either too tight or not tight enough. Reasons for inadequate restraint vary between slaughter plants and restraining boxes (WLR Report 395).



***Figure 4****: example of a NAWI restrainer for cattle in a slaughter plant*

In most cases, the head restrainer or chin lift was more or less released during bleeding, before unconsciousness was determined. However, slight release of the restrained head, sometimes appeared to improve bleeding out quality.
Results from the inversion of cattle confirmed that restraint as such is stressful. No treatment was found to be significantly more stressful than another (upright or inverted at either 90 or 180 degrees).
Both studies indicated that good quality restraint of cattle is feasible, but special attention has to be given to type and size of the restraining box, expedience of technique and equipment to animal type and skilful operators should also be properly instructed and trained well.

### 7.2.2 Bleeding

The quality of the blood flow is of major importance to induce unconsciousness. The lack of oxygen due to the loss of blood flow to the brain causes the onset of insensibility (Gregory 2004, Mellor & Littin 2004). The quality of the cut, the location of the neck cut and obstructions after the cut can have a negative influence on blood flow. After the carotid arteries have been severed, the cut ends provide an escape route by which blood drains from the circulation. Hence it is likely that most of the blood flowing towards the brain in the vertebral arteries will flow towards the rostral stump of the severed arteries (Johnson et al. 2005). Occlusion of the rostral stump of the carotid artery would block the exit route of vertebral blood and could result in the maintenance of brain perfusion. A relatively common occurrence is characterised by retraction and contraction of the elastic portion of the arterial wall and thrombus formation around the severed end of the vessel, called 'ballooning' (Gregory et al. 2006, Gregory et al. 2010, Gregory et al. 2012). This hampers the blood flow. It is suggested that a neck cut close to the jaw at C1-C2 will limit the occurrence of ballooning (Gregory et al. 2012; Grandin, 2012). In the observational study (WLR Report 395) for the majority of the animals (83%) blood flow was classified as good and most animals were cut at C1-C2.
Reasons for poor or hampered bleeding in the studies described in this report appeared to be diverse. In the study on veal calves (WLR Report 405) hampered bleeding occurred due to physical obstruction, for example when animals cut in an upright position dropped down into a hanging position on the front of

the restrain box. In other occasions ballooning, false aneurisms, blood clots and inappropriate cutting hampered bleeding.
Bleeding quality in general is good when animals are restrained correctly and the neck cut is performed well, i.e. both carotid arteries severed and hampered bleeding is prevented.

### 7.2.3 Unconsciousness

The survey in Dutch slaughter plants (WLR Report 395) together with the study on validation of indicators to determine unconsciousness in veal calves (WLR Report 405) showed that the indicators to assess unconsciousness at 40 s as specified in the Dutch covenant should be applied are un-applicable or should be applied with caution:

- Rhythmic breathing was difficult to evaluate because observation of breathing movements is influenced by the restraining method and also by severance of the trachea.
- Reaction to a threat was difficult to observe in a substantial percentage of the animals. Some of the animals close their eyes and keep them closed in response to the neck cut. Sometimes eye responses can’t be observed due to the amount of blood flowing over the eyes.
- Spontaneous blinking was absent in 68% of the animals, present in 20% and impossible to observe in 12% of the animals.
- The righting reflex could not be assessed due to full body restraint during slaughter without stunning and is therefore not an adequate indicator to assess unconsciousness.
- Reaction to a pain stimulus was absent in the majority of animals even when other reflexes were still present and animals had been classified as conscious based on EEG activity.

Palpebral and corneal reflexes were absent in only 10% of the animals at 40 s post neck cut. These reflexes were often present after unconsciousness had been defined based on EEG activity.
The results from the study to validate indicators to determine unconsciousness in veal calves shows that even in the group of calves that showed good and fast bleeding out more than 50% were conscious at 40 s after the neck cut. Similar results have been previously described by Blackmore & Newhook 1983, Daly et al. 1988, Johnson et al. 2014, Lambooij & Kijlstra 2009).
It can be argued that larger animals require more time to bleed out to lose consciousness compared to smaller animals. Therefore it is predictable that for cows and heavy bulls more animals will still be conscious at 40 s after the neck cut (observations project ‘Borest’ and Figure 5).

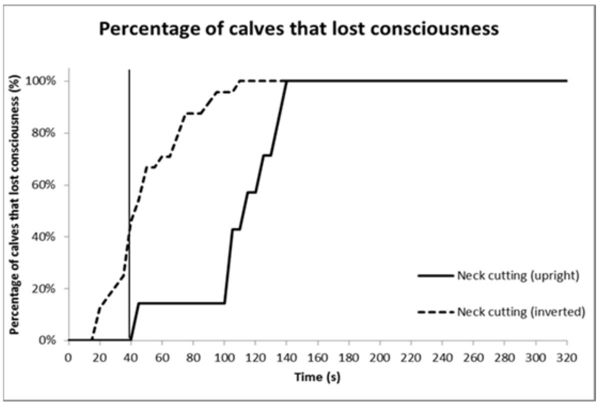



***Figure 5**: Percentage of calves that lost consciousness over time after non-stunned slaughter in an upright position (n=7) and inverted position (n=25). Vertical black line represents the percentage of calves that had lost consciousness at 40 s.*

Based on these results it can be questioned if cattle, veal calves as well as mature animals, can meet the criteria as set in the covenant on slaughter without stunning. Therefore the expediency of cattle to slaughter without stunning in terms of animal welfare is questionable.

# 8 Conclusions

The aim of this research project was to address and draw conclusions on the following topics regarding animal welfare during slaughter without stunning in cattle and sheep:

*1. Current practice of slaughter without stunning in The Netherlands*
Observations at Dutch slaughter plants identified a large variation in restraining methods for sheep, goats and cattle. More than half of the observed restrained animals did not meet legal requirements.

*2. Validation of indicators to assess (un)consciousness*
Indicators (e.g. reaction to threat; reaction to a pain stimulus; righting reflex; spontaneous eyelid reflex; rhythmic breathing) to determine consciousness during slaughter without stunning proved to be difficult to apply due to the layout of the slaughter plant or the method of restraint.
Due to the full body restraining of the animal it is not possible to observe the righting reflex and rhythmic breathing. They are therefore unsuitable indicators for assessment of unconsciousness in animals slaughtered under these conditions. Furthermore, rhythmic breathing cannot be observed at the mouth or nostrils, since it is masked by the severed trachea.
The majority of the animals did not respond to a pain stimulus following the neck cut though they were assessed as conscious based on EEG activity. The threat reaction was often difficult to assess due to the amount of blood flowing over the eyes of inverted animals and was also observed to be absent in EEG-conscious animals.
The cornea- and eyelid reflex were often present when calves were considered unconscious, based on EEG-activity. Presence of the eyelid- or cornea reflex at 40 s therefore does not provide a reliable indication of consciousness. Absence of the eyelid-, or cornea reflex on the other hand reflected unconsciousness, but both reflexes appeared distinctly conservative indicators of unconsciousness since both were lost after animals had lost consciousness (based on EEG) for up to two minutes. In sum, neither the reaction to threat nor the reaction to a pain stimulus, the righting reflex, spontaneous eyelid reflex and rhythmic breathing can be used in cattle and sheep as reliable indicators of unconsciousness under practical slaughterhouse conditions.

*3. Difference between restraint in an upright and inverted position of cattle*
Restraining and rotation is stressful for cows. It has been shown that there is an acute stress effect from rotation, and repeated restraint and rotation proved to be more stressful to a cow. It can therefore be argued that the negative experience of restraint and rotation is retained. However, although distinct indicators such as whites of the eyes or blood pressure, do show differences between treatments, there is no overall indication that any of the treatments (upright, rotation of 90 degrees or 180 degrees) is more stressful to the animal than the other treatments.
During restraining the quality of the bleeding appeared to be hampered more frequently than during inverted restraint, resulting in a significant delay in the induction of unconsciousness. When considering animal welfare aspects, stress due to restraint itself along with the effects of the restraining method should be taken into account.

*4. Reducing time to loss of consciousness after neck cut*
The time to loss of consciousness after neck cutting varied strongly between animals and species. However, the majority of sheep in the different studies were considered unconscious well before the permitted 40 seconds. However, the majority of cattle, were considered to be conscious long after the 40 s.
Since bleeding out quality in the majority of the animals was good, it can be concluded that changing the neck cut procedure [as done in practice?] will not reduce the time to loss of consciousness. A significant reduction in period of consciousness after cutting can be achieved by post cut captive-bolt stunning.

*5. Expediency of animals subjected to different slaughter procedures to animal welfare*

Expediency of different slaughter procedures to animal welfare was evaluated in the context of the agreement on slaughter without stunning in The Netherlands. Important criteria regarding animal welfare are; 1) that animals can be restrained according to regulations, 2) that animals are unconscious at 40 seconds post neck cut.

1) Although there are large differences in the quality of restraint and although the quality of restraint is, in practice, is often inadequate, it is concluded that it is technically possible to restrain all categories of animals adequately and in accordance with legal requirements.
2) It is concluded that the vast majority of sheep and goats are unconscious before 40 seconds post neck cut and therefore meet requirements.
The majority of cattle, veal calves, cows and bulls are still conscious at 40 seconds post neck cut. There is a large variation in time to loss of consciousness and consciousness can last up to 2 minutes.

# 9 Acknowledgements

The collection of information of current practices would not have been possible without cooperation of the NVWA. Furthermore, collaboration with the slaughter plants was essential to collect the data as shown in this report.
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To explore the potential of nature to improve the quality of life

Wageningen UR Livestock Research
P.O. Box 338
6700 AH Wageningen
The Netherlands
T +31 (0)317 480 10 77
E info.livestockresearch@wur.nl
www.wageningenUR.nl/livestockresearch

Livestock Research Report 417

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